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NEPHROLOGY FORUM

Filtration function in glomerulonephritis

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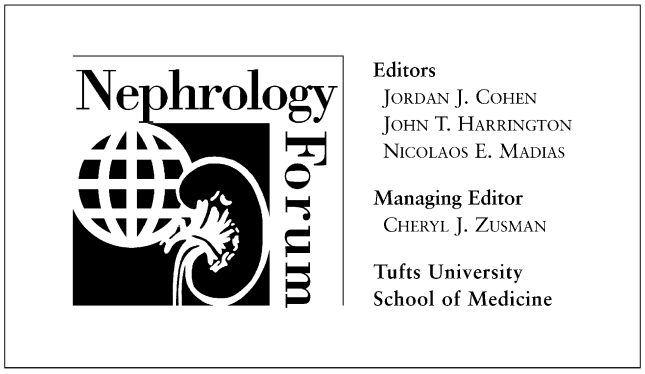

CASE PRESENTATION

A 53-year-old man was first evaluated in the outpatient clinic of the American University of Beirut Medical Center 2 years ago. He had been in good health until 13 years ago, when he developed leg swelling and "hypertension and proteinuria"; records from that time were unavailable. The patient was living in Saudi Arabia. Renal biopsy at that time revealed membranous nephropathy. The serum creatinine was reported as "normal." The patient reports being treated with the "Ponticelli protocol" [1], with complete remission of symptoms.

The patient remained free of symptoms and without treatment until 7 years ago, when swelling in his legs returned while he was in Freiburg, Germany. A physician there performed a second renal biopsy, which contained 17 glomeruli, none of which was sclerosed, and all of which demonstrated typical light and electron microscopic features of membranous nephropathy. The urinary protein excretion (Upr) was 20 g/24 hours, and the serum creatinine was 1.2 mg/dL. Blood pressure was 140/100 mm Hg. Between 7 and 2 years ago, the patient received treatment with mycophenolate mofetil (CellCept) for 4 years, a repeat of the "Ponticelli protocol," and, when seen by us 2 years ago, he was receiving cyclosporine A (175 mg/day). Steroids had been discontinued because the patient had a severe psychosis characterized by aggressive behavior and other untoward effects, including skin changes and osteoporosis of the spine. His serum creatinine also had risen gradually, to 1.4 mg/dL 3 years ago, and was 1.9 mg/dL when we examined him 2 years ago. The Upr had fluctuated markedly between 7 and 2 years ago, with a maximal value of 14 g/24 hours 3 years ago.

Two years ago, his Upr was 1.74 g/24 hours and his blood pressure was 130/90 mm Hg. The rest of the physical examination was unremarkable. His medications included (in addition to cyclosporine), enalapril (10 mg twice a day), hydrochlorothiazide (25 mg/day), and atorvastatin (20 mg/day). No changes in therapy were advised, and he was asked to return for follow-up in 2 months. He was not seen until 7 months later, at which time his serum creatinine was 2.0 mg/dL, Upr was 1.72 g/24 hours, and the blood pressure had increased to 160/110 mm Hg. Losartan was added at 100 mg/day. Between February and December of last year, cyclosporine A was continued but at a lower dose, and marked improvement was noted in the Upr, which fell to 0.19 g/24 hours; his blood pressure was lower than 130/80 mm Hg. The serum creatinine was stable at 1.9 to 2.0 mg/dL.

The patient was lost to follow-up until 3 months ago, when he was again seen in clinic for elevated blood pressure (150/100 mm Hg), increased serum creatinine (2.4 mg/dL), and increased Upr (1.5 g/24 hours). Over the subsequent 2 weeks, serum creatinine increased rapidly to 4.4 mg/dL and Upr to 4.3 g/24 hours. Tissue from a third renal biopsy performed last month contained 12 glomeruli, three of which were sclerosed. The remainder were characterized by advanced membranous nephropathy on electron microscopy, but severe proliferative changes also were present in all glomeruli, with crescent formation in three of them. In seven glomeruli, trichrome stain revealed moderate to advanced fibrotic changes. Titers for anti-glomerular basement membrane (anti-GBM) antibodies were positive. The patient was admitted to the hospital for intravenous cyclophosphamide therapy and plasmapheresis; therapy reduced his serum creatinine to 2.8 mg/dL. He is still under close monitoring for active proliferative glomerulonephritis complicating underlying, long-standing membranous nephropathy.

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Key words: end-stage renal disease, proteinuria, podocyte injury.

DISCUSSION

Dr. Kamal F. Badr (*Professor and Chair, Department of Internal Medicine, Attending Nephrologist, American University of Beirut Medical Center, American University of Beirut, Beirut, Lebanon*): "This book is written because we have come to the conclusion that the present day treatment of patients with renal [glomerular] disease is inadequate and sometimes dangerous." Thomas Addis in *Glomerular Nephritis*, 1948.

Fifty-six years of clinical and basic research later, Addis's statement remains valid, and the clinical course of this patient reflects the reasons: we still have no definitive therapy for one of the most common forms of glomerular disease in adults, membranous nephropathy [2]. It also reflects our poor understanding of the factors that govern the natural progression (or lack thereof) of this disease, the frustrations and complications of the various immunosuppressive therapies, and the lack of substantial decisive evidence favoring a particular course of treatment over another in a given patient. In the case of this patient, whose professional life entails moving among several locations (Saudi Arabia, Germany, and Lebanon), this created even greater confusion, since caregivers have no clear internationally recognized treatment guidelines. The lack of consensus on therapy for glomerulonephritis is by no means unique to membranous nephropathy. Equally frustrating to physicians and patients is the management of IgA nephropathy, primary proliferative glomerulonephritides, membranoproliferative glomerulonephritis, and many subtypes of lupus nephritis and other secondary forms of glomerulonephritis [2]. Approximately 1.0 to 1.5 million individuals worldwide carry the diagnosis of glomerulonephritis [3]. Primary and secondary glomerulonephritis are identified as the underlying cause of end-stage renal disease in 15% to 20% of patients currently undergoing dialysis in the United States [3]. This percentage has remained relatively constant over the past 25 years despite the steadily increasing total number of dialysis patients; this supports the notion that, since the days of Addis, we have failed to achieve significant progress in treating glomerulonephritis [3].

In all forms of glomerulonephritis, the only available treatments today are immunosuppressive agents (Table 1), the use of which is associated with numerous complications and untoward effects, including nephrotoxicity with agents such as cyclosporine (as in this patient). In this Forum, I aim to address the reasons underlying the disappointing lack of progress in affecting the course of glomerulonephritis despite several decades of research into the pathophysiology and therapy of these diseases. I shall address these questions based on the fundamental principle that our aim as nephrologists is primarily to arrest or reverse the two major functional morbidities associated with glomerulonephritis: proteinuria and renal insufficiency [reduction in glomerular filtration rate (GFR)]. I will attempt to answer six questions: *(1)* What is glomerulonephritis? *(2)* How does glomerulonephritis lead to proteinuria? *(3)* What are treatment strategies for proteinuria in glomerulonephritis? *(4)* How does glomerulonephritis lead to reduced GFR? *(5)* What are treatment strategies for preserving GFR in glomerulonephritis? *(6)* Is renal biopsy helpful in determining treatment outcomes?

What is glomerulonephritis?

A clear definition of glomerulonephritis is warranted for the purpose of this discussion because several forms of glomerular disease associated with proteinuria and progressive renal insufficiency, such as focal segmental glomerulosclerosis, are often labeled as glomerulonephritis, when in fact little or no inflammatory reaction is identified on biopsy. The term "glomerulonephritis" is therefore restricted to diseases in which glomeruli contain inflammatory cells, namely, polymorphonuclear leukocytes (PMNs), tissue macrophages, or lymphocytes, beyond the number expected in normal glomeruli ("resident" macrophages) [4]. In the vast majority, immune deposits (antigen-antibody complexes) are demonstrable in mesangial, subendothelial, or subepithelial locations in affected glomeruli [2]. In some cases, such as membranous nephropathy, inflammatory changes cannot always be readily identified on routine light microscopy. Careful analysis of glomeruli from such patients, however, clearly identifies increased numbers of activated glomerular macrophages [5, 6]. The presence of infiltrating leukocytes is usually a result of chemoattractant stimuli, predominantly the C5a component of complement [2, 5, 6] and leukotriene B4 [7] which, in turn, are released following antigen-antibody complex deposition and the initial wave of PMN infiltration within the glomerulus [2, 5–7]. Thus, glomerulonephritis is essentially an auto-destructive inflammatory disease, much like auto-immune arthritis, vasculitis, or colitis. This being the case, does the fact that the patient was diagosed as having "membranous" glomerulonephritis provide additional useful information for prognosis or therapy?

As Figure 1 illustrates, auto-immune antibody–mediated glomerulonephritis can be viewed conceptually as consisting of three distinct phases: initiation, amplification, and effector cell activation (which mediates functional deterioration). In the first phase, the "spark" that initiates the reaction must occur. In membranous glomerulopathy, this consists of antibody binding to antigen on the glomerular epithelial cell [2, 5], but it could be any of a number of other sites or routes of immune complex deposition (see Fig. 1). In some cases, such as acute post-infectious glomerulonephritis or experimental anti-GBM antibody-mediated injury, antibody

Table 1. Currently available therapies for glomerulonephritis

Disease	Immunosuppressive therapy	Other therapy
Membranous glomerulonephritis	Corticosteroids, cyclophosphamide, azathioprine, cyclosporine A, mycophenolate mofetil, anti-C5 antibodies, anti-B cell antibody (rifuximab)	Intravenous Ig
Mesangial proliferative glomerulonephritis	Corticosteroids, cyclophosphamide	Dipyridamole, aspirin, warfarin
IgA nephropathy	Corticosteroids, cyclophosphamide, cyclosporine A, azathioprine, mycophenolate mofetil	Intravenous Ig, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, fish oil
Crescentic glomerulonephritis	Corticosteroids	Plasmapheresis
Anti-glomerular basement membrane glomerulonephritis	Corticosteroids, cyclophosphamide, cyclosporine A, azathioprine	Plasmapheresis
Pauci-immune crescentic glomerulonephritis	Corticosteroids, cyclophosphamide, azathioprine	
Lupus glomerulonephritis	Corticosteroids, cyclophosphamide mycophenolate mofetil, azathioprine, cyclosporine, tolerance-inducing molecules (LJP-394), lymphoid irradiation, stem cell transplant	Intravenous Ig, thromboxane A_2 antagonist
Wegener's vasculitis	Corticosteroids, cyclophosphamide, mycophenolate mofetil, cyclosporine A	Plasmapheresis
Polyarteritis	Corticosteroids, cyclophosphamide	

binding leads to an acute PMN infiltrate that immediately mediates functional deterioration (proteinuria, decreased GFR) [2, 7–10]. In the majority of clinically encountered glomerulonephritides, however, acute PMN infiltration is not apparent on renal biopsy. Rather, a chronic reaction is set in motion (amplification) in which complement components, T cells, cytokines, autacoids, lipid mediators, and other biologically active molecules secreted by indigenous and infiltrating cells participate to various degrees in antigen recognition and amplification as well as perpetuation of the initial insult [2]. As Figure 1 shows, activation of indigenous glomerular cells, infiltrating leukocytes, and platelets mediates the functional consequences that underlie clinical disease. The extent of proteinuria and the magnitude and the time course of reduction in GFR are highly variable, not only among the various pathologic classifications of glomerulonephritis, but also among individual patients within each disease category [2].

Our current "naming" (classification) of glomerulonephritis into the various pathologic entities recognized in clinical nephrology is based on unrelated and seemingly random parameters, which include: the *site* of immune-complex deposition and cellular proliferation ("mesangioproliferative" glomerulonephritis), descriptive pathology of *affected structures* ("membranous," or "membranoproliferative," indicating basement membrane thickening with or without cellular proliferation, respectively), the *ultrastructural target* of circulating antibodies ("anti-GBM" disease), the *type of reactive antibody* ("IgA nephropathy"), the *underlying systemic disease* ("lupus nephritis"), or the predominant anatomic *distribution* of inflammatory changes ("focal segmental glomerulonephritis"). The lack of significant progress in our treatment of glomerulonephritis using these definitions of disease can be attributed, in part, to *the lack of any specific and reproducible clinical implications for these definitions of disease.* Are these pathologic expressions of glomerulonephritis sufficiently distinct in their clinical outcomes to warrant their classification as separate disease entities, or are they merely a reflection of variability in the permutations of immune-complex formation and localization against the complex structural background of the glomerulus? In almost all these disease entities, clinical outcome is determined not by the name we give to the inflammatory process, but by the severity and the time course of injury, both of which are extremely variable and unpredictable, as illustrated in "membranous" or "IgA" nephropathies. After so many years and so little progress [3], is it time to question the validity, utility, and clinical relevance of paradigms which are, in essence, descriptive pathologic variations on the monotone theme of auto-immune inflammation (in a complex micro-anatomy) translated, without sufficient justification, into distinct clinical entities?

How does glomerulonephritis lead to proteinuria?

Proteinuria is a feature of all forms of glomerulonephritis. In fact, proteinuria is a feature of almost any glomerular disease, whether inflammatory or not. We now realize that the reason for this lies in the exquisitely sensitive predisposition of the glomerular podocyte to injury, and the vital role of structural and functional integrity of the podocyte in preserving the permselectivity of the filtration barrier to the passage of albumin and macromolecules [11–30]. In particular, it is now clear that the slit diaphragm, which connects podocyte foot processes to each other as they overlay the basement membrane, is the principal locale of fluid flux into

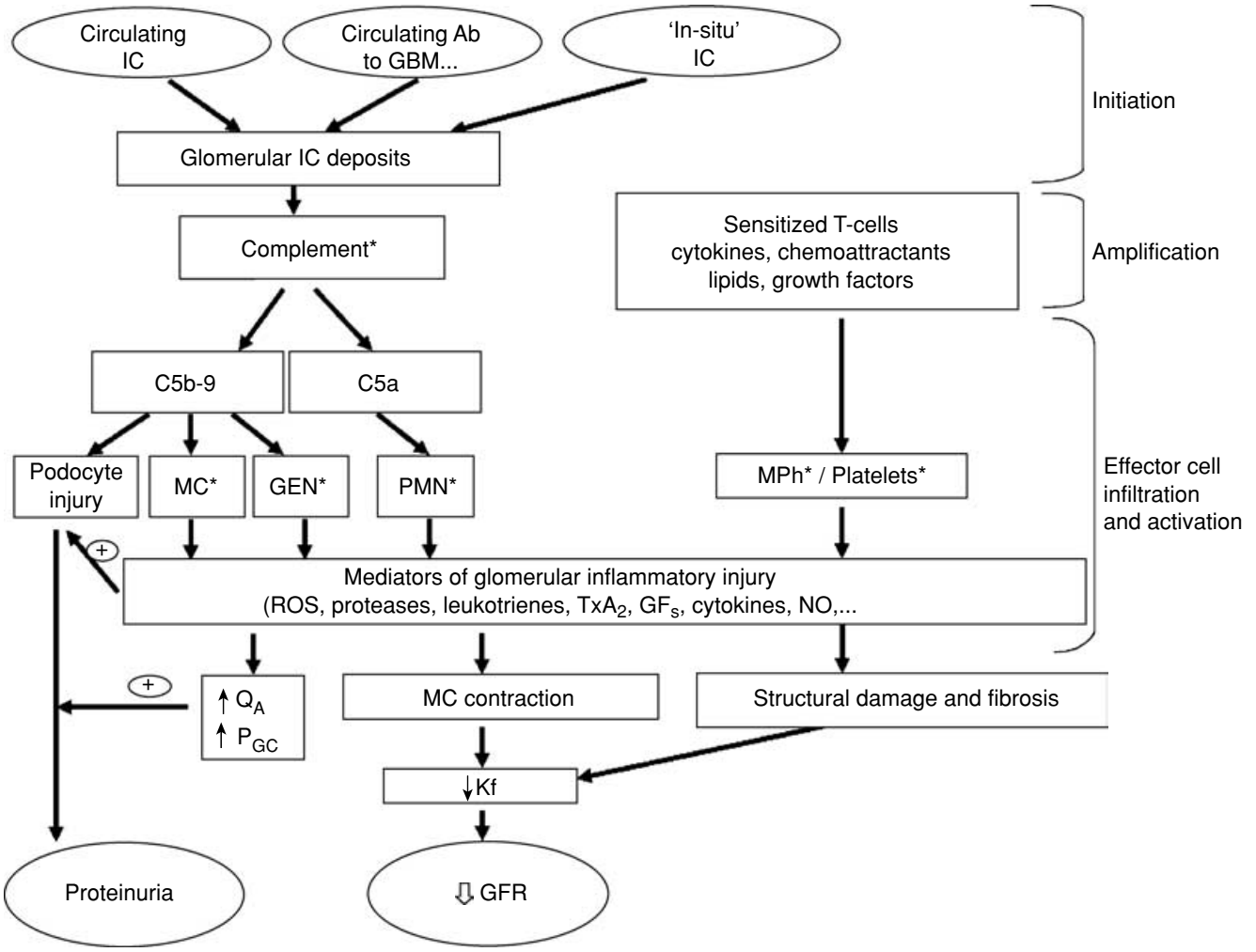

Fig. 1. The pathophysiology of proteinuria and of decreased glomerular filtration rate (GFR) during glomerulonephritis. Podocyte injury results from direct complement binding and from activation (*) of glomerular and infiltrating cells, leading to proteinuria, the magnitude of which is amplified by elevations in single nephron plasma flow rates (Q_A) and glomerular capillary pressure (P_{GC}). The fall in GFR is attributed to reductions in the filtering surface area (and possibly other) components of the glomerular capillary ultrafiltration coefficient (Kf), which can result from reversible mesangial cell (MC) contraction, irreversible structural damage, or a combination of both. Abbreviations are: ROS, reactive oxygen species; TxA_2, thromboxane A_2; GFs, growth factors; NO, nitric oxide; GEN, glomerular endothelial cells; PMN, polymorphonuclear cells; MPh, macrophages; IC, immune complex; GBM, glomerular basement membrane.

Bowman's space. Injury, dysregulated function, or activation of surface receptors of the podocyte, its foot processes or slit diaphragm molecular composition, results in proteinuria and nephrotic syndrome in several forms of congenital and acquired proteinuric disorders [11–37]. Several excellent recent reviews describe the rapid increase in our body of knowledge on the biology and pathobiology of the glomerular podocyte, its genetic development, molecular equipment, anatomic organization, and role in proteinuric disorders [11–13, 23]. A summary of slit diaphragm and podocyte-associated proteins that have been implicated in mediating congenital and acquired nephrotic syndrome and/or proteinuria is presented in Figure 2. Judging by the exponentially expanding literature on the podocyte, we can expect many more years of effort and an increasing number of publications devoted to this fascinating cell.

Appreciation of the central role of the podocyte and the slit diaphragm in glomerular molecular sieving function inevitably led to its study in the context of glomerular inflammation. In the particular case of membranous nephropathy and its experimental models (active and passive Heymann nephritis), the podocyte itself is the target of the antibodies that initiate the process of glomerular inflammation [2, 4–6]. The target antigen is localized to the foot processes, to which antibodies gain access through as-yet-incompletely defined pathways [2]. The binding of antibody to podocyte cell membrane, the resulting activation of complement, and the subsequent inflammatory reaction and macrophage activation all

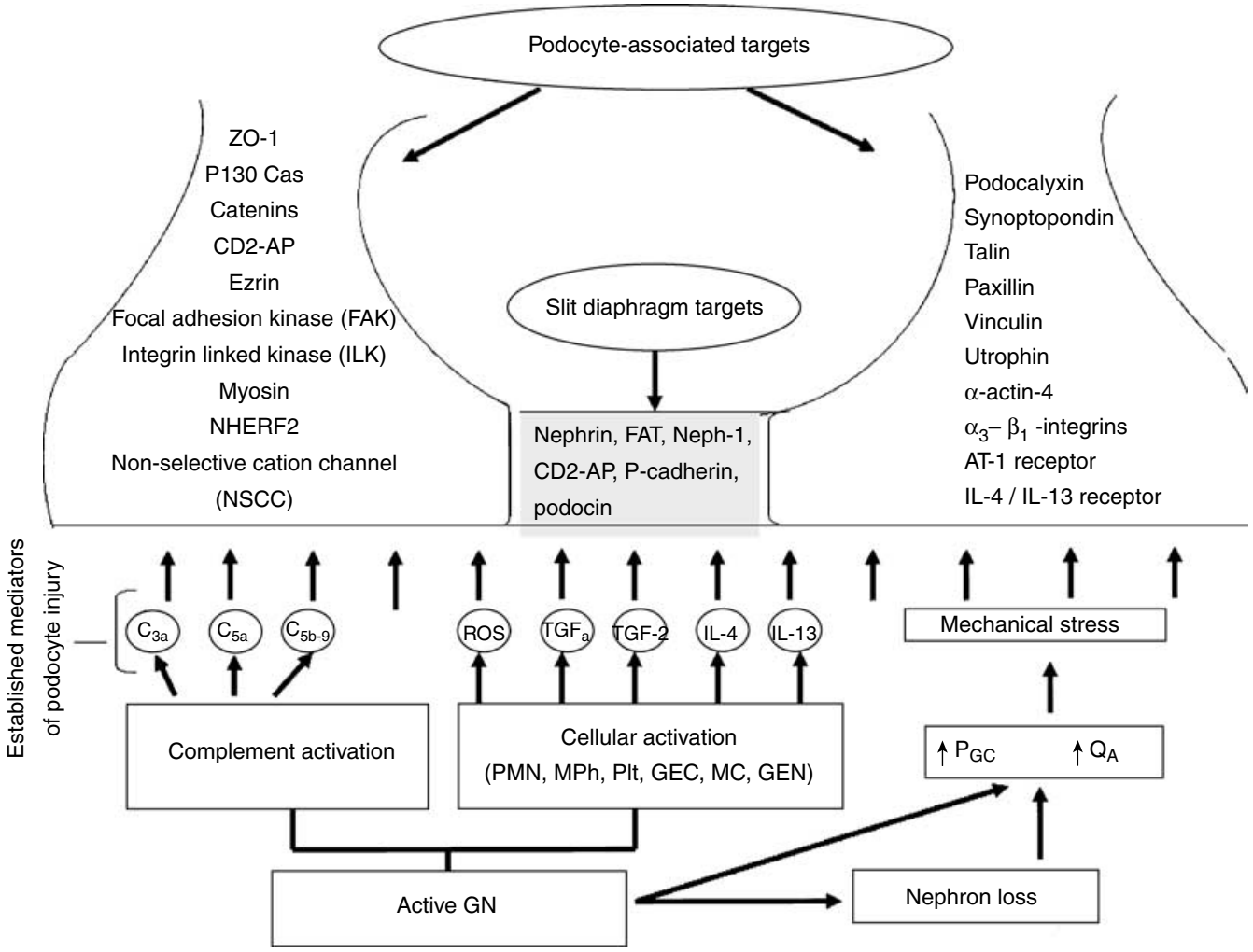

Fig. 2. Podocyte injury in glomerulonephritis. Summary of the current literature on established complement and cell activation-dependent mediators of podocyte injury and target proteins on the podocyte and the slit diaphragm which have been associated with congenital or acquired proteinuria [11–33]. Note that mechanical stress imposed by elevations in single nephron plasma flow rate (Q_A) and glomerular capillary pressure (P_{GC}) results from vasoactive locally released products of cellular activation [4–10], as well as the adaptive responses to progressive nephron loss. See Figure 1 and text for abbreviations.

conspire to disrupt podocyte integrity and produce massive proteinuria [2, 4–6, 18, 24, 29]. In approximately 10% of patients, long-standing smoldering inflammation can increase in severity with the development of anti-GBM disease, as occurred in the patient described here, and can lead to rapid deterioration in GFR and robust inflammatory changes on biopsy (including "crescent" formation) [2]. In all other forms of glomerulonephritis, podocyte injury and proteinuria result from the consequences of inflammatory reactions occurring in the vicinity of the podocyte or even in the mesangium [11–13]. Two principal pathways have been described as mediating podocyte injury during acute and chronic glomerulonephritis: direct attack by complement (particularly the C5b-9 complex) or, more significantly in the chronic phase of injury, disruption of podocyte functions by autacoids of the inflammatory response released following activation of PMNs, macrophages, mononuclear cells, and indigenous glomerular cells [11–37] (Fig. 1). The latter pathway involves numerous classes of biologically active molecules including reactive oxygen radicals [11], proteases [12, 13], leukotrienes, thromboxane and other vasoactive lipids [5, 38, 39], cytokines [31–33], growth factors [30], nitric oxide [26, 27, 34], and others [35]. In several cases, these locally generated mediators have been linked to specific molecular targets within or on the surface of podocytes [11–13] (Fig. 2). Taken together, these observations provide intriguing insight into the multiple mechanisms of proteinuria in various forms of inflammatory injury in the glomerulus but provide little hope for specific targeted interventions. It is clear from the totality of podocyte-focused research that, in the control of glomerular macromolecular sieving, foot processes and slit diaphragms bear an exquisitely delicate anatomic and functional

relationship to the underlying basement membrane, and that even subtle abnormalities in the micro-environment can affect these relationships in a manner that invariably seems to cause loss of the normal restriction to albumin filtration [11–37]. That the GFR is normally close to 180 liters/day results in dramatic amplification of abnormal single-nephron sieving function and leads to overt proteinuria.

Acute elevation of glomerular capillary pressure in the normal glomerulus does not produce significant proteinuria but will do so when sustained for prolonged periods (hyperfiltration nephropathy) [40]. In the presence of glomerular inflammatory injury, however, lowering of intraglomerular pressure often is associated with significant amelioration of proteinuria. This decrease suggests that in the presence of pre-existing pathology in podocyte structure or function, elevated (or even normal) glomerular pressure exacerbates abnormal protein passage [2]. In addition to elevated capillary pressure, proteinuria can be exacerbated by diminished rates of single-nephron plasma filtration rates, likely as a result of prolonged contact time and diffusion-governed transmembrane protein passage [41]. In experimental models of chronic glomerulonephritis, single-nephron plasma flow rates (Q_A), glomerular capillary pressure (P_{GC}), and net transcapillary hydraulic pressure difference (ΔP) were elevated [5, 8–10, 39, 42], in keeping with clinical observations [43, 44]. Increased single-nephron pressure and flow might represent significant mechanical stress to glomerular mesangial and epithelial cells (podocytes); this stress, in turn, is associated with secondary pathologic alterations in these cells [11, 45], further exacerbating proteinuria and nephron loss (Figs. 1 and 2).

What are treatment strategies for proteinuria in glomerulonephritis?

Glomerulonephritis has received little attention from the pharmaceutical industry as a distinct target for research and drug development. This is not surprising, as the "market size" for these diseases worldwide is comparatively small, and their pathophysiology complex. Therapeutic tools available to the nephrologists are entirely "borrowed" from other disciplines and mainly comprise immunosuppressive agents, of which corticosteroids continue to be the most commonly used (Table 1).

As I said earlier, proteinuria during glomerular inflammation is a result of direct complement-mediated and/or inflammation-associated podocyte injury. Complement components and products of inflammation collectively target a multiplicity of cell surface, slit-diaphragm, and intracellular podocyte structures (Fig. 2). It is therefore unlikely that a single anti-proteinuric agent aimed at any of the myriad potential targets of injury on podocytes will prove useful in completely abrogating proteinuria during glomerulonephritis. Thus, at present, the treatment of proteinuria in patients with active glomerulonephritis primarily depends on the control of inflammatory injury through the use of immunosuppressive agents. Some additional tools are in various stages of validation as effective interventions for reducing proteinuria in human glomerulonephritis: angiotensin antagonists, complement antagonists, and 5-lipoxygenase-activating protein antagonists.

In keeping with the notion that elevated intracapillary pressure in the glomerulus exacerbates proteinuria, success in reducing urinary protein excretion in glomerulonephritis has been achieved through the use of angiotensin antagonist therapy, including protocols combining angiotensin-converting-enzyme (ACE) inhibitors (ACEi)s and angiotensin receptor blockers (ARBs) [2]. In fact, prolonged use of intensive angiotensin antagonism has been associated with improved prognosis in some forms of glomerulonephritis (such as IgA nephropathy) [2]. Proteinuria in and of itself is considered of pathologic significance in mediating downstream (tubule) tissue damage, so it is possible that intensive combined (ACEi and ARB) angiotensin antagonism therapy will prove increasingly effective in the prevention of disease progression.

Complement-neutralizing antibodies (anti-C5 antibodies) have been developed for human use in attempts to limit infarct size during acute myocardial infarction [46, 47]. Such therapies, however, have not been tested in human glomerulonephritis.

Biologically active products of the 5-lipoxygenase pathway of arachidonic acid metabolism have been implicated in mediating proteinuria and reductions in GFR in several forms of experimental glomerulonephritis [5, 7, 8; reviewed in 39, 48–50]. In the first trials in human disease, 11 patients with active glomerulonephritis were treated for 4 days with twice-daily doses of an orally active antagonist of 5-lipoxygenase activating protein (FLAP), MK-591, a key nuclear membrane-bound protein required for 5-lipoxygenase activation and leukotriene synthesis [51]. Short-term therapy with MK-591 reduced proteinuria in these patients by 50% without altering systemic hemodynamics, GFR, or renal plasma flow rates. Analysis of the fractional clearance of polydispersed dextrans revealed that treatment with MK-591 caused a selective improvement in the passage of large ($\geq$50 Å) dextrans without affecting the handling of smaller dextrans, indicating an improvement in glomerular size selectivity [51].

How does glomerulonephritis reduce GFR?

Progressive reduction in the GFR is the most serious consequence of chronic glomerulonephritis. In membranous nephropathy as well as in IgA nephropathy, lupus

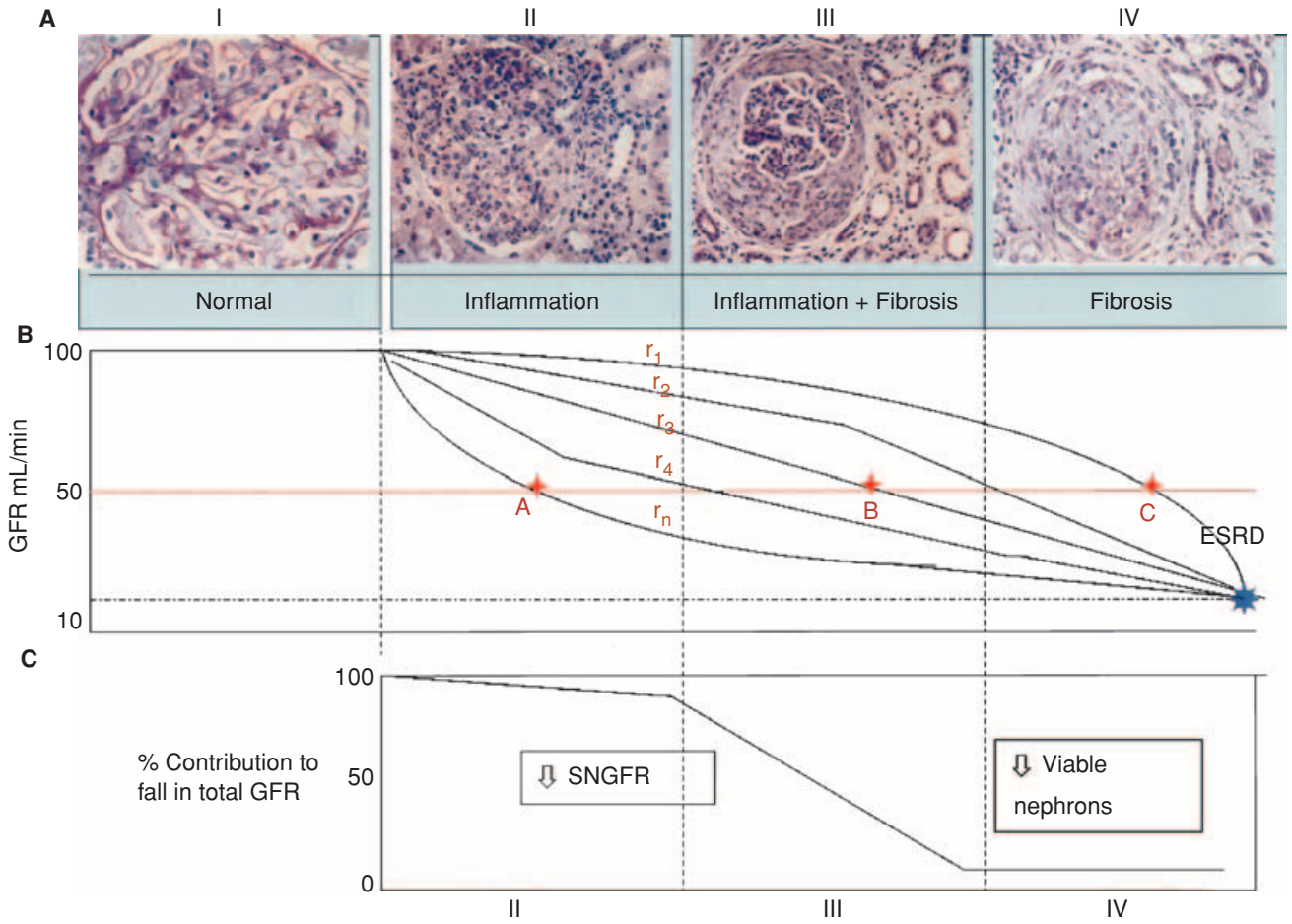

Fig. 3. (*A*) Individual glomeruli in patients with glomerulonephritis fall into one of four histopathologic patterns: normal, inflammation without fibrosis, a combination of inflammation and fibrosis in the same glomerulus, and fibrosis with loss of architectural integrity. (*B*) The rates at which glomerular filtration rate (GFR) is lost can vary infinitely and are described by a family of curves (r_1 to r_n). A 50% reduction in GFR, however, may result solely from reversible reductions in Kf (A), irreversible reductions in Kf (C), or a combination of both (B) (See Fig. 2). (*C*) Dynamic evolution of the principal mechanism underlying the fall in total GFR over the course of glomerular inflammatory injury as individual glomeruli transition from stage II to stage IV. Essential to the choice of therapy (anti-inflammatory, anti-fibrotic, or none) is valid quantitative measurements of the proportion of nephrons in each stage (see hypothetical profiles described in text). ESRD is end-stage renal disease; SNGFR is single-nephron GFR.

nephritis, and all other forms of classically recognized histopathologic disease entities, patients can survive for years with relative preservation of GFR, can experience a slow progressive reduction in GFR, or can lose renal function rapidly over the course of days to weeks [2] (Fig. 3B). So far, few specific prognostic indexes can reliably predict the course of renal functional deterioration in any of the various nephritides. In addition to inter-individual variability, single-nephron GFR (SNGFR) varies widely among nephrons in each patient, as evidenced by the extreme variability in histologic involvement of nephron units on biopsy (Fig. 3A). Estimation of GFR from serum creatinine reflects overall integration of nephron function and does not provide information as to the underlying mechanism.

Clearly, GFR is compromised by the presence of inflammation in the glomerulus. In rats fed an arachidonic acid–deficient diet from the time of weaning until adulthood, immune-complex deposition fails to elicit a cellular response, and glomerular functions (including GFR) are completely preserved [52]. These observations support a significant body of evidence that assigns central roles for cyclo-oxygenase and lipoxygenase products of arachidonic acid in sustaining and perpetuating histopathologic and functional alterations in glomerulonephritis [5, 7, 8, 38, 39, 48–51]. Studies using glomerular micropuncture have defined the mechanisms underlying the fall in GFR during acute inflammation as being primarily a reduction in the glomerular capillary ultrafiltration coefficient (Kf) [4–10, 38, 39]. The determinants of Kf are the glomerular transcapillary hydraulic permeability (k) and the capillary surface area available for filtration (S) [53]. The latter is in turn a reflection of two components: structurally intact glomerular architecture (that is, absence of

fibrosis, collapse, or disruption of structure-function relationships between capillaries and corresponding Bowman space), and the state of contraction or relaxation of mesangial cells, which determines the number of open capillary loops in a particular glomerular tuft [53]. Loss of structure is associated with irreversible loss of surface area, whereas mesangial cell contraction is potentially reversible [8] (see Fig. 1). These formulations represent a simplified approach to explaining the fall in Kf during glomerulonephritis; the exact mechanisms could be more complex. As-yet poorly understood factors (other than structural damage and reduction of open capillary loops) could underlie the fall in Kf and might be reversible with anti-inflammatory therapy [14]. Thus, in a particular patient, reduced SNGFR in all involved glomeruli might reduce overall GFR but maintain an intact complement of nephrons (as in acute post-infectious glomerulonephritis). More commonly, a combination of reversible reductions in SNGFR in some nephrons and an irreversible loss of filtration surface area due to disruption/destruction of structural elements in other nephrons together decrease GFR (Fig. 3).

Reversibility of the fall in GFR during acute glomerulonephritis is a crucial determinant of the indications for immunosuppressive therapy. As Figure 3 illustrates, the success of therapy in preserving GFR in unaffected glomeruli, or in reversing the reduction in SNGFR in inflamed but as-yet non-fibrosed glomeruli, can only be accurately predicted if the percent contribution of the functional (reversible) fall in Kf to the overall reduction in GFR is known. At present, we assign, based on examination of renal biopsy, what is known as an "activity versus chronicity score," to determine reversibility of the fall in GFR. Based on this "measurement," the clinician decides on the administration, or the withholding, of immunosuppressive therapy [2, 54]. In my discussion of treatment strategies for the fall in GFR, I shall argue that *this approach does not in fact provide a faithful or a reproducible reflection of the extent of reversibility of the loss of filtration function.* Without a reliable estimate of the contribution of inflammation versus fibrosis to the overall loss of GFR, the outcomes of therapeutic interventions cannot be judged.

What are treatment strategies for preserving GFR in glomerulonephritis?

In the patient presented here, who carried the diagnosis of membranous nephropathy for 18 years, GFR was initially preserved, but gradual loss of renal function ensued in the latter part of his disease as the serum creatinine level rose to 1.4 and then 2.0 mg/dL. In the latest phase of his disease, the GFR was reduced markedly and rapidly, as his glomerular lesion transformed into a severe proliferative form of glomerulonephritis. The impact of immunosuppressive treatment on GFR was difficult to evaluate in the chronic phase of the disease, but intravenous cyclophosphamide (and possibly plasmapheresis) were clearly associated with rapid improvement in GFR following transformation of the lesion to a proliferative crescentic glomerulonephritis. Acute substantial improvements in GFR by the use of pulse steroids, intravenous cyclophosphamide, or other measures in patients with acute or subacute proliferative glomerulonephritis illustrate that a significant portion of the fall in GFR is due to reversible reductions in Kf, mediated by locally released vasoactive and cytotoxic products of the inflammatory reaction. In experimental models of glomerular injury, a wide range of mediators have been implicated as underlying the fall in Kf during glomerular inflammation [4–10, 14, 26, 38, 39, 42–44, 48–52, 55]. At present, however, the only available clinical therapeutic tools are drugs that non-specifically reduce the number or activation status of inflammatory leukocytes and/or indigenous glomerular cells (Table 1). Whereas immunosuppressive (and potentially anti-complement) therapies aim at arresting injury and restoring GFR by reversing reductions in SNGFR in glomeruli with "active" lesions, therapies targeting *glomerular fibrosis* might become available in the future, such as agents aimed at neutralizing the biologic actions of vascular endothelial growth factor (VEGF) [56]. Additionally, intriguing results in experimental animals suggest that "high-dose" angiotensin antagonist therapy might reduce and reverse matrix deposition and fibrosis [57]. Future choices among anti-inflammatory versus anti-fibrotic therapies for preserving GFR during glomerulonephritis, however, would still require a reliable profiling of nephronal distribution of "activity versus chronicity."

Is renal biopsy helpful in determining treatment outcomes?

At first glance, it might seem reasonable to assume that, because glomerulonephritis involves nephrons in a random fashion, a random sample, no matter its size, should reflect the true distribution of active versus fibrotic glomeruli in a particular patient. The margin of error, however (that is, the deviation of the estimated proportion of normal, proliferative, partially fibrotic, and globally fibrotic glomeruli from the true distribution of these categories in the total nephron population) increases as the sample number decreases, and thus is unacceptably high for the number of glomeruli usually obtained on routine renal biopsy (Table 2).

Let us consider two hypothetical patients (A and B) with membranoproliferative glomerulonephritis. Both present with similar clinical findings of active urinary sediment, hypertension, proteinuria, and azotemia (serum creatinine, 3.5 mg/dL). Patient A has sustained severe

Table 2. Margins of error for 90% to 99% confidence intervals[a]

Sample	Confidence			
	90%	95%	98%	99%
A				
10	22.59	26.84	31.90	35.33
20	15.98	18.98	22.56	24.98
30	13.04	15.50	18.42	20.40
50	10.10	12.00	14.27	15.80
125	6.39	7.59	9.02	9.99
250	4.52	5.37	6.38	7.07
500	3.20	3.80	4.51	5.00
1000	2.26	2.68	3.19	3.53
2000	1.60	1.90	2.26	2.50
4000	1.13	1.34	1.60	1.77
B				
10	26.09	30.99	36.84	40.79
20	18.45	21.91	26.05	28.85
30	15.06	17.89	21.27	23.55
50	11.67	13.86	16.48	18.24
125	7.38	8.77	10.42	11.54
250	5.22	6.20	7.37	8.16
500	3.69	4.38	5.21	5.77
1000	2.61	3.10	3.68	4.08
2000	1.84	2.19	2.61	2.88
4000	1.30	1.55	1.84	2.04

[a]Sample size is varied from 10 to 4000, when determining the distribution of four variables (for example, glomeruli in stages I to IV) in a poluation. Sample B is the same as in sample A if histologic classification is reduced to only two stages (for example, "active" versus "chronic" only).

glomerular inflammation that resulted in rapid destruction and fibrosis of more than 75% of his nephron population. Active injury persists in 20% of the remaining nephrons, with advanced but incomplete fibrosis present in 15%. The remaining 5% of nephrons are normal. Thus, in accordance with the staging proposed in Figure 3, his nephron profile would be: stage I: 5%, stage II: 5%, stage III: 15%, and stage IV: 75%. Patient B, on the other hand, is in a phase of acute active inflammation, with only 10% completely fibrosed glomeruli (stage IV), 5% partially fibrosed (stage III), 80% actively undergoing proliferative injury (stage II), and the remaining 5% still intact (stage I).

At present, renal biopsies performed in both cases will yield information that carries a very high probability of being completely erroneous regarding the true distribution of nephron pathology. The reason is the very high statistical probability that the number of glomeruli sampled by biopsy (at best 20 to 30) will not reflect the true distribution of active versus chronic injury in the *total* nephron population of both kidneys (Table 2). Human kidneys contain anywhere from 400,000 to 1.4 million glomeruli, with marked inter-individual variability [58, 59]. If one seeks to determine with any degree of confidence the distribution of four variables (in this case stages I to IV) in nephron populations above 20,000 to 30,000, then the following statistical assumptions and formulae apply:

Assumptions: (*1*) Glomeruli fall randomly into one of four stages (Fig. 3). (*2*) Each stage takes on a binomial distribution (that is, there is some probability that a given glomerulus is either in, say, stage I, or it is not). (*3*) There is mutual exclusivity among the four stages (that is, a glomerulus in stage I cannot also be in stages, II, III, or IV). (*4*) The sum of the probabilities of glomeruli being in stages I, II, III, and IV equals 100%.

Formulae: Any estimate of the proportion for one stage (for example, stage I) takes on a margin of error with a certain degree of confidence. For example, if one samples 250 nephrons and estimates the proportion of glomeruli in stage I to be 25% based on that sample, one could be 95% confident of a margin of error of 5.37% (Table 2). This means that had one performed this procedure 100 times, 95 of those times one would be within 5.37% of the true proportion. Since all the assumptions listed here hold for patients with glomerulonephritis, one can simply use the standard method of determining a margin of error as one would for any binomial problem according to the following equation:

$$\text{z of } (1 - \text{alpha}/2) \times \sqrt{[(\text{p}(1 - \text{p})/\text{n})]}$$

where "z of (1-alpha/2)" is the 1-alpha/2 percentile of the standard normal distribution Z, p is the probability one is seeking to estimate (such as the proportion of nephrons in each stage), and n is the sample size. Based on this equation and assuming equal proportions ($P = 0.25$) for each stage (when operating under uncertainty this is the most unbiased assumption), Table 2 provides margins of error based on a range of sample sizes and confidence values. For example sizes less than 30 (most renal biopsies contain fewer than 30 glomeruli) it becomes clear that the margin of error in estimating the proportion of

glomeruli in any particular stage of injury becomes unacceptably high even if one is seeking a confidence limit of only 90% (Table 2). If one is to obtain a greater than 95% confidence of being within less than 5% margin of error, one would need to sample somewhere between 250 and 500 glomeruli!

These calculations should not be surprising, because it is reasonable to expect that 10 to 20 glomeruli can hardly be a statistically representative sample for a population of 0.4 to 1.4 million. Lacking this information, however, all assumptions regarding the success or failure of therapeutic interventions in altering the course of renal functional deterioration in glomerulonephritis would necessarily be faulty. Add to this uncertainty in defining the true cause of the fall in GFR the relatively low number of patients in most clinical trials focusing on a particular form of glomerulonephritis, it is no wonder that the results of clinical trials are so frequently contradictory and inconclusive [2] *The lack of significant progress in reducing the overall incidence of glomerulonephritis as a cause of ESRD* [3] *therefore might be due in large measure to erroneous assumptions regarding the true cause of reduced GFR in particular patients, such that the response (or lack thereof) to therapy is rendered open to major errors in interpretation.* For example, if patient A in the hypothetical example given here had been biopsied and, say, 10 glomeruli obtained, the margin of error in estimating activity versus chronicity would have been greater than 25% (Table 2)! If the sample happened to be biased in favor of activity, and the patient had been treated with aggressive immunosuppressive agents, not only would he have suffered the toxicity of treatment, but the protocol employed might have been deemed ineffective in restoring or preserving GFR in membranoproliferative glomerulonephritis when in fact the major true cause of the fall in Kf and GFR (partial or total fibrosis in more than 90% of nephrons) was missed. Conversely, patient B might have been denied treatment based on its failure in Patient A or because of a sampling error in the opposite direction.

CONCLUSIONS

The number of patients with glomerulonephritis worldwide is increasing. Randomized controlled trials have been difficult to perform and interpret, and the results are all too often contradictory [2, 54]. Consequently, available pharmacologic therapies, principally non-specific immunosuppressive drugs, have made limited impact on overall disease prevalence and on kidney and patient survival. It might be time for us to re-assess classifications and paradigms that have formed the basis of our current clinical approaches to this group of diseases. It may prove more useful for preserving glomerular sieving and filtration function to approach therapeutic strategies (and hence clinical trials) based on structural determinants of functional abnormalities (inflammation versus fibrosis) rather than specific histopathologic disease entities. In parallel, the determination of predominant mechanisms of reduced overall filtration rate should employ statistically valid approaches to nephron profiles that reflect the true distribution of inflammation versus fibrosis in the nephron population of the two kidneys. This is a major challenge, as renal tissue sampling to obtain the required number of glomeruli for valid statistical analysis (95% confidence and margins of error of less than 5%) (Table 2) might not be clinically feasible. A more fruitful approach possibly would be to search for urinary markers of disease activity (for example, complement proteins) that are quantitative, easy to measure, and reflect ongoing inflammatory injury integrated over the entire nephron population. Hopefully, the challenges presented in this Forum will lead to rethinking of current strategies for diagnosing and treating glomerulonephritis, so that the prevalence of these diseases, and their contribution to the incidence of end-stage renal disease, will be reduced.

QUESTIONS AND ANSWERS

DR. JOHN T. HARRINGTON (*Dean Emeritus, Tufts University School of Medicine, USA*): Approximately 40 years ago, Kark, Pirani, and others [60–62] showed that renal biopsies containing approximately 5–10 glomeruli were satisfactory for determining the specific histopathologic type of renal disease, so we have relied heavily since then on the biopsy for categorization and treatment. What do we substitute for the biopsy to obtain the more dynamic information you correctly call for?

DR. BADR: The studies you refer to are indeed pioneering works that established the usefulness of biopsy for determining histopathologic subtypes of renal disease. Interestingly, in the classic 1958 study analyzing the results of 500 biopsies by Kark et al [60], the majority of samples contained 5 to 14 glomeruli. I am not calling for any substitute to the biopsy to define the type of glomerular disease in a particular patient. As you point out, there is a need for more dynamic information regarding the degree to which changes in GFR are reversible. What I argue here is that, for purely statistical reasons, the number of nephrons obtained is inadequate for that type of analysis. The evolving nature of the disease renders single determinations of "activity" at one point in time of little value in long-term follow up and management.

DR. HARRINGTON: Specifically, what do we use to clinically measure "Kf"? Also, how do intrarenal inflammation and intrarenal fibrosis contribute to its reduction?

DR. BADR: If we could measure Kf, which at this time is not clinically feasible, or if one were to obtain a quantitative measure of what Brian Myers calls "glomerulopenia" [14], we would obtain information which is not dissimilar from that related by serum creatinine. In other

words, we would measure the degree of impairment of glomerular function, but that would provide no insight as to its mechanism. The question remains as to the degree of reversibility; what proportion of the fall in Kf is due to inflammation versus fibrosis? The problem is that the contribution of inflammation or fibrosis to the fall in Kf varies from nephron to nephron, from day to day, and from patient to patient. This extreme variability demands a type of measurement that can be repeated frequently and that reflects the degree of active inflammation, even as that component of the fall in Kf varies over time. How to achieve this is the challenge for all of us. A starting point might be to analyze urinary proteomics (urinomics) of pooled samples from large numbers of patients with active glomerular disease, without regard to histopathologic subtypes, and see whether we can discern some predictors of subsequent clinical deterioration.

DR. K. L. GUPTA *(Postgraduate Institute of Medical Education and Research, Chandigarh, India):* Dr. Badr, you have shown us very lucidly the role of inflammation in glomerular injury and consequent renal dysfunction. Which of the anti-inflammatory/immunosuppressive drugs would you have preferred to use in this patient who had membranous glomerulonephritis at presentation?

DR. BADR: At present, no evidence supports changing our current approach to the treatment of newly discovered membranous nephropathy. The use of any immunosuppressive therapy is guided by the extent of functional impairment and the activity of the urinary sediment. I believe the majority of physicians would still use corticosteroids as the mainstay of initial therapy for this disease.

DR. GUPTA: What is the role of ACE inhibitors and ARBs in reducing proteinuria and preserving GFR in membranous nephropathy?

DR. BADR: Clear evidence indicates that angiotensin antagonism decreases proteinuria in IgA nephropathy and other forms of inflammatory glomerulonephritis, including the use of combined ACE inhibition and angiotensin receptor blockade [2, 63]. It would be of interest to examine the role of ARBs alone in proteinuric disorders, particularly in view of the potential role of podocyte AT1 receptor activation during some forms of proteinuric glomerular diseases.

DR. NATHAN LEVIN (*Renal Research Institute, New York, NY, USA*): Do you think that the use of physical approaches, such as comparative measurements of renal temperature or imaging techniques [for example, multislice magnetic resonance images (MRI)] could provide an integrated assessment to distinguish inflammation from fibrosis?

DR. BADR: I am not aware of any data supporting the notion that integrated measurements of renal temperature are useful in assessing the degree of inflammation. Your second idea, however, in regard to MRI or other imaging techniques might be applicable. Imaging is one of those modalities that might identify targets which, for example, are present uniquely in inflamed glomeruli. These are ideas worth exploring.

DR. JAN J. WEENING (*Academic Medical Center, Amsterdam, The Netherlands*): Considering the success of cytotoxic and immunosuppressive treatment in severe necrotizing glomerulonephritis, is there not a clear need for accurate diagnosis and classification of glomerular disease, as illustrated in this patient with two different types of glomerulonephritis?

DR. BADR: There is no question that kidney biopsy, which clearly defines the specific diagnosis of the underlying glomerular disease, is of paramount importance in guiding therapy. There is always the occasional patient who presents a diagnostic dilemma that can only be resolved through a biopsy, and the identification of specific entities guides therapeutic approaches. The patient presented here, as you correctly point out, illustrates the importance of repeated biopsies in identifying transformation of one disease form to another and necessitating a different therapeutic strategy. Pathologists must continue to play a central role in defining and characterizing glomerular inflammatory diseases; asking them to quantitatively define the mechanisms underlying overall reductions in GFR from the examination of kidney biopsies with 10 or 15 glomeruli, however, is not statistically or biologically valid or feasible.

DR. WEENING: Glomerular perfusion and capillary pressure are important determinants of glomerular immune-complex deposition and accumulation, as well as glomerular permeability to albumin and other plasma proteins. Lowering blood pressure also affects compensatory hyperfiltration in remaining nephrons and protects the tubulointerstitial compartment [63, 64]. For example, unilateral renal artery stenosis protects the kidney from immune complex accumulation in membranous glomerulopathy and lupus [65, 66]. Would therefore aggressive lowering of blood pressure (for example, to 100/60 mm Hg) not be a very sensible way of treating the membranous glomerulopathy in this and other patients?

DR. BADR: I am a strong believer in the profound influence of physical forces on the biology of glomerular cells. We have published some of the earliest work on the effects of such forces on mesangial cell structure and function in vitro [45]. I have referred to the established effects of sheer stress on podocyte biology. Your suggestion for aggressive blood pressure lowering is indeed a reasonable one, and it already enjoys support from the data on proteinuria.

DR. MAHBOOB LESSAN-PEZESHKI (*Tehran University of Medical Science, Tehran, Iran*): What are the basic mechanisms of isolated hematuria in some forms of immune-mediated glomerulonephritis such as IgA nephropathy? It seems that if the size of pores in the membrane is large enough to allow passage of cells (such

as red blood cells), why can't proteins, which are much smaller, pass?

DR. BADR: It is important to recognize that the "pores" I referred to in relation to the passage of proteins are not actual physical pores. Rather, they are theoretical constructs that fit experimental data supporting the presence of a "shunt" pathway in the glomerular filtration barrier. This "shunt" pathway selectively increases the proportion of the filtrate passing through large molecular size pathways or "pores." The passage of red blood cells from the glomerular capillary to the intratubular lumen relates to the elevated pressures in the capillary and consequent forced entry of the red cells through the GBM, and leads to red cell membrane tears and dysmorphic morphology of red cells, a criterion that has been used to distinguish glomerular hematuria on examination of the sediment. This passage occurs at discrete sites and does not represent a generalized leakiness of the GBM. It might occur even in the absence of significant proteinuria, as in some patients with IgA nephropathy.

DR. SALIM MUJAIS (*Baxter Healthcare Corporation, Mc Gaw Park, IL, USA*): You suggested that the change in our therapeutic paradigm requires a "summation profile" that is developed from urinomics. There are precedents of using a "summation response" in nephrology to guide therapy, such as the "captopril test," or modified positron emission tomography (PET) [67]. Considering the expected long time gap in satisfying the urinomic requirement, do you think there would be value in developing a clinical test, such as the response to the Merck product (MK591) that you used in your *Kidney International* paper [51]? Such a test would be non-invasive, widely applicable, and could be repeatedly applied in the same patient as either a natural history map or a measure of therapeutic response.

DR. BADR: That would certainly be a welcome tool, if it were available. The Merck product, which is a 5-lipoxygenase activating protein antagonist, did indeed provide a nearly uniform reduction in urinary protein excretion rates when given for 4 days, even though the magnitude of the fall in proteinuria was much more evident in patients with significantly elevated baseline values [51]. Whether MK591 can be used as a "clinical summation response" test to determine the potential reversibility of functional derangements remains to be seen. Your idea, however, is exactly the kind of approach we should be exploring: clinical measurements that can distinguish patients likely to benefit from aggressive immunosuppressive or other therapies.

DR. PAULOSE P. THOMAS (*Belhoul Specialty Hospital, Dubai, United Arab Emirates*): You mentioned that you would wait until the GFR dropped before you would treat a patient who had membranous nephropathy with immunosuppression (which is the current recommendation). Don't you think that by the time the GFR dropped in a slow and steady manner fibrosis already would have set in and it might be too late to treat the patient? The reason why many patients, including the patient just discussed, did not achieve remission might be because we lost an opportunity to treat the patient when his condition was in inflammatory mode and possibly reversible.

DR. BADR: That is exactly the point of my presentation. If we had a better way of monitoring the course of this patient in a manner that would allow us to predict reductions in GFR before the creatinine has risen, then it might be possible to initiate treatment in a rational and timely manner prior to significant increases in serum creatinine. This can only be achieved, however, by monitoring urinary or other parameters that track, in a dynamic and faithful manner, ongoing reversible reductions in GFR.

DR. RAJESH RAJ (*Welcare Hospital, Dubai, United Arab Emirates*): What is the role of inflammation within the kidney in patients with essential hypertension, proteinuria, and diminished renal function? Are there studies on this?

DR. BADR: The role of inflammation in hypertension-associated glomerular sclerosis is gaining increasing attention. Earlier in this meeting, Dr. Weening presented elegant formulations linking glomerular sclerosis in patients with metabolic syndrome and atherosclerosis to a common underlying systemic inflammatory condition. Evidence that atherosclerosis is an inflammatory condition is now widely accepted, and the possibility that some forms of focal segmental sclerosis are the glomerular counterpart of atherosclerotic vascular injury deserves further investigation.

PROF. JAMAL ALWAKEL (*King Saud University, Riyadh, Saudi Arabia*): What is the role of renal tubule and interstitial tissue injury in glomerular disease? Could treatment failures in glomerulonephritis be due to a lack of attention to factors related to tubular and interstitial tissue?

DR. BADR: There is no doubt that the degree of interstitial injury is a determinant of clinical outcome in patients with primary glomerular disease. Recent evidence points to a potential role for misdirected filtration outside Bowman's space leading to initiation of periglomerular injury and fibrosis [2, 11, 12]. Other mechanisms initiating tubular injury during glomerulonephritis include filtration of proteins, such as IgG and oxidized low-density lipoprotein (LDL), which interact with brush border and other luminal cell surface target molecules, and lead to the initiation of secondary tubulointerstitial injury [2]. These processes accelerate and amplify loss of nephron structure and lead to progressive loss of function, but the primary pathology remains in the glomerulus, and that is the logical target for therapy.

DR. JOHN DIRKS (*University of Toronto, Toronto, Canada*): In your model, what schematic diagram would you employ to depict the course of the presented patient dividing progressive loss of GFR as due to the element of inflammation, or due to fibrosis or both to illustrate your thesis?

DR. BADR: As Figure 3 shows, a family of curves describes the possible course of renal functional deterioration in patients with glomerulonephritis. For each curve, the relative contribution of inflammation versus fibrosis would differ, but it is possible for patients to move from one curve to another with the intervention of effective therapy. I believe this model best describes the course of this patient.

DR. DIRKS: The patient had a slowly progressive chronic kidney disease, perhaps slowed by rigorous immunosuppressive treatment, and then developed a very inflammatory anti-GBM disease. What factors in membranous glomerulonephritis could make it more susceptible to anti-GBM disease?

DR. BADR: A certain percentage of patients with membranous nephropathy develop anti-GBM disease which, if not recognized, can lead to rapid irreversible loss of renal function. The prolonged course of this patient suggests that the duration of disease might be a factor predisposing to this ominous complication. The frequency of this occurrence is low, however, and no reliable predictive parameters for its development have been identified.

DR. AIDA MOUSALLI (*Saint Joseph University, Beirut, Lebanon*): Is there any place in the therapy of glomerulonephritis for statins and eicosapentaenoic acids? They are anti-inflammatory agents and have few secondary effects.

DR. BADR: It would seem reasonable to test the role of these molecules in inflammatory glomerulonephritis. As you know, fish oils have given encouraging results in IgA nephropathy [2]. Statins possess anti-inflammatory properties and also might be of benefit. I do not know of any randomized controlled trials using these agents in glomerulonephritis.

DR. BEENA UNNIKRISHNAN (*Cosmopolitan Hospital, Trivandrum, India*): What is the rationale behind the Ponticelli regimen? Why was it used twice for this patient?

DR. BADR: The Ponticelli protocol was designed to combine steroid-based therapy with immunosuppressive agents so as to reduce the doses of both [1]. Its use twice in this patient reflects the choice of his treating physicians at the time and is not based on the results of controlled trials demonstrating efficacy. It does illustrate, however, the lack of clear guidelines in the treatment of this as well as other primary glomerulopathies.

DR. UNNIKRISHNAN: Would you suggest a blanket therapy for all patients with glomerulonephritis using statins, antioxidants, and omega fatty acids?

DR. BADR: I do not think such an approach, reasonable at it sounds, can be justified at this time. It awaits the results of randomized controlled studies.

DR. SAMIR H. AL-MAILO (*King Fahd Hospital of the University, Alkhobar, Saudi Arabia*): In patients with acute glomerulonephritis, the outcome can range from complete resolution to progressive scarring and end-stage renal disease. In your opinion, what factors determine the outcome of acute glomerulonephritis?

DR. BADR: The determinants of the outcome of inflammatory injury (resolution versus progression) are the subject of much research and controversy. As you know, children with post-infectious glomerulonephritis nearly always recover completely, whereas adults with the identical disorder often progress due to unrelenting inflammatory injury [2]. In some way, age modifies our capacity to put a stop to unwanted auto-destructive inflammation. "Stop" signals to inflammation have been linked through the work of Serhan and his colleagues to endogenous lipid-based mediators [see 68 and 69 for comprehensive reviews of "endogenous anti-inflammation"]. Prominent among these pathways is the 15-lipoxygenase (15-LOX) enzyme [70], which is induced in macrophages of nephritic glomeruli in experimental animals [71] and catalyzes the transformation of arachidonic acid to lipoxins and other anti-inflammatory lipids. Introduction of the human 15-LOX gene into rat kidneys also ameliorates glomerular immune injury [72]. The capacity to mount a robust anti-inflammatory response is in turn genetically determined and linked, in part, to interleukin-4 and 13 (IL-4 and IL-13) synthesis by Th2-lymphocytes [71, 73, 74]. IL-4 and IL-13 are uniquely capable of inducing de-novo expression of 15-LOX in human leukocytes [73]. There is thus evidence supporting the notion that the extent of induction of endogenous anti-inflammatory responses dictates the outcome of glomerular immune injury [68–74]. Clinically, predictors of a poor outcome are the severity of the reduction in GFR on diagnosis, and the magnitude of proteinuria [2].

DR. HARRINGTON: What urinary biomarkers could be used at present, and what is the "gold standard" to which we would compare these newer more dynamic markers?

DR. BADR: Currently there are no reliable urinary markers for ongoing renal inflammation. The National Institutes of Health has expressed interest in this issue, and a recent workshop was dedicated, in part, to developing the science of urinomics in the setting of glomerulonephritis. The nephrology community will eagerly anticipate the results and recommendations of this workshop as well as future conferences and research efforts to help identify urinary markers that provide an integrated measurement of active inflammation in glomeruli.

DR. WEENING: Several centers currently study renal biopsies by gene transcription and proteomics analysis in addition to classical histopathology, including

immunofluorescence and electron microscopy. The outcome of renal tissue RNA, proteomics, and in situ hybridization in relation to histopathology as well as urine and plasma genomics and proteomics might yield interesting new data and could change the contribution of plasma, urine, and biopsy data to the diagnosis.

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